NOTE TO THE FILE

January 25, 1996

Subject: Glufosinate Tolerant Corn

Keywords:

Corn, *Streptomyces hygroscopicus*, Glufosinate ammonium tolerant, Herbicide tolerant, *bar*, Phosphinothricin Acetyltransferase (PAT), *bla*.

Background

In a submission dated November 17, 1995, Dekalb Genetics Corporation provided summary information to support the safety and nutritional assessment of its new glufosinate herbicide tolerant corn line DLL25, which was previously known as B16. The firm initiated its consultation in a submission dated July 5, 1995.

Intended Effect and Food/Feed Use

The intended technical effect of this genetic modification of corn is to confer tolerance to the herbicidal compound, phosphinothricin (glufosinate ammonium). Corn grain is primarily used for animal feed, but also has human food uses. Human food products which are milled from grain include starch, corn syrup, corn oil, dextrins, corn meal, flour, grits, and breakfast cereals. Corn is also used in the distillation and fermentation industries to produce ethyl and butyl alcohol, acetone, and alcoholic beverages. The foliar part of corn plants is commonly used for the production of silage, an animal feedstuff. Corn may also be used to produce non-food products.

Dekalb indicates that corn line DLL25 has been modified to express the *bar* gene obtained from *Streptomyces hygroscopicus*. The *bar* gene encodes the phosphinothricin acetyltransferase (PAT) enzyme, which reportedly confers tolerance to the herbicide glufosinate ammonium by specifically acetylating phosphinothricin.

Molecular Alterations and Characterization

The novel genetic material contained in line DLL25, was inserted into A188 x B73 type II callus line cells using microprojectile bombardment. The transformation vector, pDPG165, was derived from pUC19 plasmid. Dekalb reports that the *bar* gene, its associated regulatory sequences and a truncated form of the *bla* gene were integrated into the corn genome. The intact *bla* gene encodes for ß-lactamase, an enzyme which degrades penicillin-type antibiotics. The integration event is described as a single copy of *bar* at one site in the long arm of chromosome 3. Based on data obtained from Southern blot analyses, Dekalb states that the genetic insert is composed of an intact *bar* gene, one incomplete copy each of the 35S promoter and *bla* gene, and two partial copies of

transcript 7 of the *Agrobacterium tumefaciens* T-DNA (Tr7) 3' region inserted in opposite orientation with respect to each other.

The firm reports that DNA sequence analysis of the ß-lactamase gene cloned from the DLL25 line demonstrated that the *bla* gene is truncated at the 5' region base pair 568 of the 858 base pair coding sequence. Results from Northern and Western blot analyses are reported by the firm to confirm that line DLL25 does not express ß-lactamase. In addition, the firm indicates that the *bla* gene is under the control of a prokaryotic regulatory sequence and thus, would not be expected to be expressed in plants. When the truncated *bla* gene present in DLL25 was transferred to *E. coli*, the firm states that the bacterial cells remained sensitive to ampicillin (100 mg/ ml) and did not produce detectable ß-lactamase based on Western blot analysis (sensitivity level of below 10 ng).

The *bar* gene is reported to be completely sequenced. The gene inserted into line DLL25 has had the initiation codon modified from GTG to ATG to conform with gene expression in plants. Based on genetic segregation and Southern blot analyses, the firm reports that the DLL25 insert has remained stable for over 10 generations. Dekalb reports that herbicide tolerance segregates in a 1:1 pattern when heterozygous DLL25 plants are backcrossed to inbreds, consistent with a single insertion site.

Expressed Protein

Dekalb states that, based on Western blot and enzymatic activity assays, the PAT protein encoded by the *bar* gene is present in both the grain and green portions of the corn plant. Dekalb reported that the PAT protein was not detected in pollen. The firm reports that PAT makes up 0.13% of total grain protein and 0.35% of total forage protein at the late milk stage of plant development. The firm indicates, however, that no PAT activity is detected after the silage fermentation process. The firm concludes that active PAT will be present in grain. but not in silage. The firm anticipates that PAT will not be present in corn oil, since proteins are water-soluble. As discussed previously, Dekalb indicates that the *bla* gene is not expressed in DLL25 corn.

PAT is stated to be heat labile with enzymatic inactivation reported to occur after incubation at 50°C for 4 hours. No enzymatic activity was detected following 24 hours of incubation at 25°C. Dekalb reports that using Western blot analysis, there was greater than a 10-fold reduction in PAT concentration after incubation at 50°C for 24 hours.

Dekalb states that PAT is highly specific for phosphinothricin and shows no reactivity with glutamate or other glutamate analogues. The firm also reports that PAT exhibited no activity with a mixture of amino acids obtained from a casein hydrolysate.

Allergenic and Toxic Potential

Dekalb conducted a protein sequence homology search for PAT. The databases were: Brookhaven Protein Data Bank; Swiss-Prot including Ancient Conserved Regions subset;

PIR; CDS translations from GenBank; Kabat Sequences of Proteins of Immunological Interest; TFD Transcription Factor Database, and Translations of select Alu repeats from REPBASE. The firm concludes that PAT from *Streptomyces hygroscopicus* exhibits no homology to known toxins or allergens. In addition, the firm reports that the *bar* gene contains no glycosylation motifs. Dekalb reports that PAT was not detectable 1 minute after placement in simulated gastric fluid (detection limit < 10 ng). However, the enzyme was reported to be stable in neutralized gastric fluid. When added to simulated intestinal fluid, PAT was not detected after 10 seconds. Based on these results, Dekalb states that "the Pat protein is unlikely to be an allergen."

In summary, the firm concludes that:

> the PAT protein will not be a macroconstituent in the human or animal diet, and will comprise at most approximately 0.13% of the total protein consumed by an animal or human if that animal or human fed exclusively on grain from corn hybrids containing the DLL25 transformation event. Furthermore, it is likely that all PAT protein that is ingested by an animal will be rapidly degraded and therefore the exposure of the animal will be minimal. We therefore conclude that the PAT protein present in DLL25 corn hybrids is not a safety concern.

Nutritional Assessment

Grain

Based on the nature of the genetic modification, it was expected that DLL25 corn would not materially differ in composition from other corn varieties. To confirm this expectation, Dekalb analyzed the nutrient composition of grain obtained from DLL25 corn hybrids and comparable controls by standard methods for moisture, fat, protein, fiber, ash, carbohydrate, and amino acids.

No statistically significant differences are reported by Dekalb in levels of moisture, fat, protein, fiber, ash, carbohydrate, and most amino acids between grain derived from the modified and control hybrids. The firm indicated that statistically lower levels of threonine, an amino acid, were present in DLL25 grain when compared to its non-transformed counterparts, but that values fell within the ranges reported in the literature for corn.

Vegetative Tissues

Compositional analyses of silage included moisture, protein, fat, fiber, ash, carbohydrate, the amino acid profile, acid detergent fiber (ADF), and neutral detergent fiber (NDF). Dekalb reports that transgenic lines differed from controls in concentrations of ADF and NDF. However, the firm states that despite these differences, values fell within ranges observed for nontransformed Dekalb germplasm.

Dekalb also examined the level of the antinutrient, phytic acid, in silages derived from

DLL25 and control hybrids. Phytic acid concentrations were determined to be less than 0.02% for both transformed and control materials. Dekalb indicates that inclusion of the novel genetic material in DLL25 corn did not affect hybrid susceptibility to attack by fungal organisms. Based on these observations, the firm concludes that mycotoxin-producing fungi will not be more prevalent on DLL25 corn than its non-transformed counterparts.

Dekalb concludes that "the nutritional value of DLL25 corn is essentially the same as untransformed counterpart corn hybrids and falls within the range normally seen in corn."

Conclusions

Dekalb has concluded that "human and animal consumption of corn lines containing the DLL25 transformation event does not constitute a safety concern." At this time, based on Dekalb's description of its data and analyses, the Agency considers Dekalb's consultation on corn grain, fodder, and silage derived from corn lines containing transformation event DLL25 to be complete.

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